

CAN383	.MASISVDQI	RKAQRANGPA	TVLAIGTANP	PTSFYQADYP	DFYFRVTKNQ
CAN24	MNHLRAEGPA	SVLAIGTANP	ENILMQDEFP	DYFVRVTKSE
HOP_VPS	.MASVTVEQI	RKAQRAEGPA	TILAIGTAVP	ANCFNQADFP	DYFVRVTKSE
Arabidopsis_CHS	MAGASSLDEI	RQAQRADGPA	GILAIGTANP	ENHVLQAEYP	DYFVRITNSE
HOP_CHS	...MVTVEEV	RKAQRAEGPA	TILAIGTATP	ANCILQSEYP	DYFVRITNSE
CAN_CHS_like	...MVTVEEF	RKAQRAEGPA	TIMAIGTATP	ANCVLQSEYP	DYFVRITNSE
CAN1069	...MVTVEEF	RKAQRAEGPA	TIMAIGTATP	ANCVLQSEYP	DYFVRITNSE
CAN383	HMTELKDKFK	RICEKTTIKK	RHLYLTEDRL	NQHPNLEEYM	APSLNTRQDM
CAN24	HMTQLKEKFR	KICDKSMIRK	RNCFLNEEHL	KQNPRLVEHE	MQTLDRQDM
HOP_VPS	HMTDLKKKFQ	RMCEKSTIKK	RYLHLTEEHL	KQNPHLCEYN	APSLNTRQDM
Arabidopsis_CHS	HMTDLKEKFK	RMCDKSTIRK	RHMHLTEEF	KENPHMCAYM	APSLDTRQDI
HOP_CHS	HKTELKEKFK	RMCCKSMIRK	RYMHLTEEIL	KENPNLCAYE	APSLDARQDM
CAN_CHS_like	HKTELKEKFK	RMCDKSMIRK	RYMHLTEEIL	KENPNLCAYE	APSLDARQDM
CAN1069	HKTELKEKFK	RMCDKSMIRK	RYMHLTEEIL	KENPNLCAYE	APSLDARQDM
CAN383	LVVEIPKLGK	EAAMKAIKEW	GQPKSRITHL	IFCSTNGVDM	PGADYECACL
CAN24	LVVEVPKLGK	DACAIAIKEW	GQPKSKITHL	IFTSASTTDM	PGADYHCAKL
HOP_VPS	LVVEVPKLGK	EAAINAIKEW	GQPKSKITHL	IFCTGSSIDM	PGADYQCAKL
Arabidopsis_CHS	VVVEVPKLGK	EAAVKAKEW	GQPKSKITHV	VFCTTSGVDM	PGADYQLTKL
HOP_CHS	VVVEVPKLGK	EAAATKAKEW	GQPKSEITHV	VFCTTSGVDM	PGADYQLTKL
CAN_CHS_like	VVVEVPKLGK	EAAATKAKEW	GQPKSKITHL	VFCTTSGVDM	PGADYQLTKL
CAN1069	VVVEVPKLGK	EAAATKAKEW	GQPKSKITHL	VFCTTSGVDM	PGADYQLTKL
CAN383	LGLSSSVKRV	MLYQ [*] QGC ⁺ HAG	GSVLRIAKDL	AENNKGARIL	TINSEITIGI
CAN24	LGLSPSVKRV	MMYQLG ⁺ CYGG	GTVLRIAKDI	AENNKGARVL	AVCCDIMACL
HOP_VPS	LGLRPSVKRV	MLYQLG ⁺ CYAG	GKVLRIAKDI	AENNKGARVL	IVCSEITACI
Arabidopsis_CHS	LGLRPSVKRL	MMYQ [*] QGC ⁺ FAG	GTVLRIAKDL	AENNRGARVL	VVCSEITAVT
HOP_CHS	LGLRPSVKRL	MMYQ [*] QGC ⁺ FAG	GTVLRVAKDL	AENNKGARVL	VVCSEITAVT
CAN_CHS_like	LGLRPSVKRL	MMYQ [*] QGC ⁺ FAG	GTVLR ⁺ LAKDL	AENNKGARVL	VVCSEITAVT
CAN1069	LGLRPSVKRL	MMYQ [*] QGC ⁺ FAG	GTVLR ⁺ LAKDL	AENNKGARVL	VVCSEITAVT
CAN383	FHSPDETYFD	GMVG ⁺ QALFGD	GASATIVGAD	PDKEIGERP	FEMVSAAQEF
CAN24	FRGSPDSLE	LLAG ⁺ QALFGD	GAAAVIVGAE	PDES ⁺ VG ⁺ ERPI	FELVSTGQTI
HOP_VPS	FRGPSEKHL	CLVG ⁺ QSLFGD	GASSVIVGAD	PDASVGERPI	FELVSAAQTI
Arabidopsis_CHS	FRGSPDTHLD	SLVG ⁺ QALFSD	GAAALIVGSD	PDTSVGEKPI	FEMVSAAQTI
HOP_CHS	FRGPN ⁺ DTHLD	SLV ⁺ QALFGD	GSAALIGAD	PIPEI.EKPI	FELVSAAQTI
CAN_CHS_like	FRGPN ⁺ DTHLD	SLVG ⁺ QALFGD	GSAALIVGSD	PIPEV.EKPI	FELVSAAQTI
CAN1069	FRGPN ⁺ DTHLD	SLVG ⁺ QALFGD	GSAALIVGSD	PIPEV.EKPI	FELVSAAQTI
CAN383	IPNSDGA ⁺ VDG	HLTEAGLVYH	IHKDVPGLIS	KNIEKSLVEA	LNP ⁺ IGISDWN
CAN24	LPNSEGTIGG	HIREAGLIFD	LHKDVPMLIS	NNIEKCLIEA	FTP ⁺ IGISDWN
HOP_VPS	LPNSDGA ⁺ IAG	HVTEAGLTFH	LLRDVPGLIS	Q ⁺ NIKSLIEA	FTP ⁺ IGINDWN
Arabidopsis_CHS	LPDSDGAIDG	HLREVGLTFH	LLKDVPGLIS	KNIVKSLDEA	FKPLGISDWN
HOP_CHS	LPDSDGAIDG	HLREVGLTFH	LLKDVPGLIS	KNIEKSLVEA	FKPLGISDWN
CAN_CHS_like	LPDSDGAIDG	HLREVGLTFH	LLKDVPGLIS	KNIEKSLNEA	FKPLGISDWN
CAN1069	LPDSDGAIDG	HLREVGLTFH	LLKDVPGLIS	KNIEKSLNEA	FKPLGISDWN
CAN383	SLFWI [*] VHPGG	PAILNAVEAK	LHLKKEK ⁺ MAD	TRHVLSEYGN	MSSVS ⁺ IFFIM
CAN24	SIFW ⁺ ITHPGG	KAILDKVEEK	LDLKKEK ⁺ FVD	SRHVLSEHGN	MSSSTVLFVM
HOP_VPS	NIFWIAHPGG	PAILDETEAK	LELKKEK ⁺ MKA	SREMLSEYGN	MSCASVFFIV
Arabidopsis_CHS	SLFWIAHPGG	PAILDQVEIK	LGLKKEK ⁺ MRA	TRHVLSEYGN	MSSACVLFIL
HOP_CHS	SLFWI ⁺ THPGG	PAILDQVESK	LGLKPEKLRA	TRHVLGEYGN	MSSACVLFIL
CAN_CHS_like	SLFWIAHPGG	PAILDQVESK	LALKTEKLRA	TRHVLSEYGN	MSSACVLFIL
CAN1069	SLFWIAHPGG	PAILDQVESK	LALKTEKLRA	TRHVLSEYGN	MSSACVLFIL
CAN383	DKLRKRSLEE	GKSTTGDGFE	WGVLF ⁺ GFGPG	LTVETIVLHS	LAN..
CAN24	DELKRKRSLEE	GKSTTGDGFE	WGVLF ⁺ GFGPG	LTVERVVVRS	VPIKY
HOP_VPS	DEMRRKQSKE	GKSTTGDGLE	WGALF ⁺ GFGPG	LTVETVVVLS	VPTNV
Arabidopsis_CHS	DEMRRKSAKD	GVATTGEGLE	WGVLF ⁺ GFGPG	LTVETVVVLS	VPL..
HOP_CHS	DEMRRKCAED	GVKTTGEGLE	WGVLF ⁺ GFGPG	LTVETVVVLS	VGI..
CAN_CHS_like	DEMRRKCVED	GLNTT ⁺ GEGLE	WGVLF ⁺ GFGPG	LTVETVVVLS	VAI..
CAN1069	DEMRRKCVED	GLNTT ⁺ GEGLE	WGVLF ⁺ GFGPG	LTVETVVVLS	VAI..

* reaction center + cavity size

Supplementary Table 3. Comparison of amino acid sequences of peptides related to polyketide synthases expressed in Cannabis glands. Amino acid residues predicted to be located in the reaction center and residues important for defining reaction cavity sized are marked with an "*" and "+", respectively. CAN24, 383, and 1069 are from derived from unigenes characterized in this study. Accession numbers: Arabidopsis CHS, NP_196897; hop VPS, BAB121202; hop CHS, CAK19319; Cannabis CHS, AAL92879.

Supplemental Table 4. qPCR analysis of select *Cannabis* unigenes.

A) Relative expression levels of selected genes in glands and leaves.

		Gland mean	Gland std. dev.	Leaf mean	Leaf std. dev.	gland over leaf	P values
DOXP synthase	Can548	1.98E-5	4.58E-6	9.21E-7	3.26E-07	21.5	0.0002
Malic enzyme	Can6	1.55E-5	3.26E-6	2.20E-7	3.99E-08	70.2	0.0001
putative Olivetolic acid synthase	Can24	2.66E-5	7.27E-6	1.59E-8	3.45E-09	1670	0.0003
Chlorophyll A/B Binding protein	Can219	1.08E-7	1.77E-8	4.40E-6	1.62E-06	0.02	0.0019
hydroperoxide lyase	Can413	1.56E-6	3.13E-7	1.71E-7	6.20E-08	9.15	0.0001
COP9 complex subunit	Can747	2.14E-8	4.77E-9	1.80E-9	1.53E-09	11.9	0.0002
AtMYB78&112	Can833	1.47E-6	2.01E-7	1.54E-9	1.04E-09	954	0.0001
beta-keto acylreductase	Can915	1.53E-5	3.11E-6	1.61E-7	4.67E-08	95.3	0.0001
Histone H2A	Can986	6.77E-7	1.24E-7	1.59E-7	2.36E-08	4.24	0.0002
Beta tubulin	Can1084	1.09E-5	5.91E-6	3.16E-6	2.14E-07	3.45	0.0397
THC synthase	Can720	4.15E-6	1.36E-6	9.50E-9	5.51E-09	437	0.0009
CHS-like protein	Can383	5.32E-6	4.03E-6	3.81E-6	2.87E-06	1.40	0.564
acyl carrier	Can498	1.26E-6	1.71E-7	8.49E-9	5.90E-10	148	0.0001
lipoxigenase	Can162	2.34E-8	7.18E-9	3.10E-7	5.38E-08	0.075	0.0001
3-oxoacyl-[acyl-carrier protein] reductase; beta-ketoacyl-ACP reductase	Can82	2.28E-6	6.71E-7	3.10E-9	4.36E-10	735	0.0005
acyl-CoA synthetase [Brassica napus]	Can70	1.18E-6	2.16E-7	5.26E-8	2.74E-08	22.5	0.0001
3-ketoacyl-CoA thiolase; acetyl- CoA acyltransferase	can94	4.17E-7	2.91E-8	6.14E-8	2.35E-08	6.79	0.0001
MYB related	can792	1.15E-7	1.65E-8	1.35E-8	6.56E-09	8.51	0.0001
AtMYB12&111	can738	1.94E-6	7.58E-8	3.31E-9	4.55E-10	586	0.0001
MYB related	can483	7.03E-8	1.65E-8	9.91E-9	2.46E-09	7.09	0.0004
Homeodomain HAT4 Arabidopsis	can227	2.86E-7	7.88E-8	8.22E-8	2.06E-08	3.48	0.0024
CAN CHS	can1069	1.86E-6	6.03E-8	8.43E-9	1.09E-09	220	0.0001
CONSTANS-LIKE3	can618	1.99E-9	1.26E-9	3.74E-7	5.04E-07	.0053	0.1903
lipoxigenase	can295	4.95E-8	1.57E-8	4.81E-8	4.71E-09	1.03	0.87

RNA was isolated from the glands and leaves of two different plants. Each sample was tested in two 2-step qPCR reactions. Thus, each mean and standard deviation is derived from four qPCR reactions as described in Materials and methods.

B) qPCR Primers used in this analysis.

Unigene	qFor	qRev
CAN548	GGT GGA TCA GTA CAT GAA TTG	GCA ATG GAT CAA GAC TGG AC
CAN6	CAA GCT CTC TCT GTC ATT TTA AGC	CGC TTT CCA TCA CAA CGG
CAN24	CAC ACA TAC ACA ACT CAT TAT TAT C	GAG TTG AGT CAT GTG TTC AC
CAN219	GGC CAC AAA CCA AAG AAG AAG	CTG TTT GGA AGC AAA CCA C
CAN413	GGC GAG CTC CTA TGC G	GCC GTA AGC GTG ACG ATA TC
CAN747	GCA AAT CGG CGA AAA TAA G	GAT GTG GTG GTT GCA TCT G
CAN833	CTC TCT CTC TCT CTC CAT C	CAA CAG TCC ATG GAC CTC
CAN915	CAT CAA CAT ATA GCT ATA TGG AGT TCC	CCA GAA TCA GAC TCA GAC CTT TC
CAN986	CCC TCC AAA ACT CGA ATC G	GAC TTC TTC CTG TCT CCG C
CAN1084	GGC GCT GAG TTG ATC GAT TC	GTA TGG TTC CAC AAC TGT GTC
CAN720	GGA CTG AAG AAA AAT GAA TTG C	GGT CGT GTT GAG TGT ATA CG
CAN383	CCG TGC TAG CCA TCG GAA C	CCC TAG CTT GGG AAT CTC
CAN498	CCCAGATCTCTCTCACTCTTG	GCG CCT AGG CTG CAT CTT TAG
CAN162	GCC GGA GGA AGC ACT TAT G	CTC CAC ACC GAT TCT TCA AAT C
CAN82	GCC CAC AAC AAT CTT CGG	CCC TGA GCT TTA ACA ACA CC
CAN70	GGA GGT TTA TGA TTG TGT GAT AAA AC	GCA CCT AAG GTG TCA TAC AAC
CAN94	CCC CAC CGA CCT AGA TAT G	CCG GTA AGC TGC GAC AAT AAC
CAN792	CCC TAA CAT TTG CCG ATC TG	CGG ATC GAC CCG TCA GTC
CAN738	GAA GAG GAT GAG ATC TTG ACC AAG	GCC TAA TGT AGA ATG CAG GTT G
CAN483	GAG CCA AAC CCA GAT AAG AAA AAT TG	GGT TCA CTA GCT CCA AAC ACA AAG
CAN227	CCC ACT TTC TCA CCA TCC AAA C	CGG AAC AAA CGA GGA GAG C
CAN1069	CCT CAA GTT TAC CAA ATA TGG T	GAG CTC AGT TTT GTG TTC ACT ATT G
CAN618	CTC CTT CTC GAA TCT CCT AC	GCC ACA ACA TCA AAA CGA C
CAN295	GAA CAC TCT ATG GAT CAA GAA C	GGA CAT GAG CTT TTG CAA G

C) Representative real time qPCR tracings. Relative fluorescence and cycle number are shown on the Y- and X-axes, respectively. Standards 1, 2, and 3 were generated by sequential 100-fold dilutions of the target fragments. Inserted into the figures are standard curves (cycle number vs. log of the dilution factor of the target fragment) and the melting temperatures of the qPCR products. BS42 and BS46 are designations for the two different plants used in the analyses.

